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# Clinical presentation, laboratory parameters, classification and flow cytometric analysis of patients of paroxysmal nocturnal haemoglobinuria in Pakistan

## Abstract

**Background:** Paroxysmal Nocturnal Haemoglobinuria (PNH) is a rare acquired clonal haematopoietic stem cell disorder that manifests as bone marrow failure, haemolytic anaemia and thrombosis.

**Objectives:** To determine the clinical presentation and laboratory parameters and to classify and perform flow cytometric analysis of PNH patients in Pakistan.

**Materials and method:** This cross-sectional study was conducted in the Department of Haematology and Department of Immunology, Armed Forces Institute of Pathology Rawalpindi from January 2013 to December 2017. Diagnosis was established by flow cytometry. Clinical presentation and laboratory parameters were assessed and the patients were classified according to the Working Diagnostic Classification of the International PNH Interest Group (I-PIG).

**Results:** A total of 43 patients of PNH were analyzed. Males were 28(65%) and 15(35%) were females. Median age was 28 years (14-48years). The most common presenting feature was pallor in 42 (98%) followed by fatigue in 40(93%) and dyspnea in 25(58%) of the patients. Twenty (47%) patients presented with anaemia alone, 8 (19%) with bicytopenia while 15 (35%) had pancytopenia at the time of presentation. Three (6.9%) patients presented with thrombosis. The median duration of disease at diagnosis was 3.8yrs. Mean haemoglobin was 7.8±2.6g/dl, mean platelet count was 94±52.8x$10^9$/l and mean ANC was 2.9±1.3x$10^9$/l. Patients were classified as classic PNH (53%), PNH in setting of another bone marrow disorder (42%) and sub-clinical PNH (4.7%). The median clone size was 60% in patients with classic PNH, 22% in patients with PNH in setting of another bone marrow disorder and 7% in sub-clinical PNH.

**Conclusion:** The most common presentation was pallor. Majority of patients were in the classic PNH subgroup.

**Keywords:** paroxysmal nocturnal haemoglobinuria, flow cytometry, clone size

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## Introduction

Paroxysmal Nocturnal Haemoglobinuria (PNH) is an acquired disorder characterized by chronic haemolytic anaemia.[1] This disorder is unique in the sense that it is an acquired disorder in which the defect is intrinsic to the cell.[2] Literally, paroxysmal means 'sudden and irregular', Nocturnal means at 'night' while haemoglobinuria means 'haemoglobin in urine'. Thus, paroxysmal nocturnal haemoglobinuria means 'sudden, irregular episodes of haemoglobin in urine at night'.[3]

It results from a somatic mutation in the phosphatidyl-inositol glycan anchor biosynthesis- class A gene (PIG-A gene) on the X chromosome.[4] The PIG-A gene product is required for the synthesis of the glycosylphosphatidylinositol (GPI) anchor.[1] As a result, the PNH stem cells and their progeny lack the GPI-anchored proteins.[5] Of these, lack of CD55 and CD59, GPI-anchored complement regulatory proteins, leads to complement-mediated intravascular haemolysis and thus, the manifestations of the disease.[6]

PNH has an incidence of 1.3 new cases per million population per year.[7] It may present at any age, being more common in young adults. Males and females are both affected equally.[8] Clinically, it has a variable presentation and may present as haemolysis, thrombosis or aplasia.[9] One of the most serious complications is thrombosis which usually occurs at unusual sites.[2] Patients presenting with Coombs negative haemolytic anaemia with moderate reticulocytosis, elevated LDH and possibly mild jaundice are investigated for PNH.[10] Traditionally, PNH was diagnosed by the sucrose lysis test and the Ham's test which demonstrated increased sensitivity of PNH RBC's to complement-mediated lysis. However, today, flow cytometry has greatly replaced these tests.[11] Flow cytometry using antibodies against the GPI-AP deficient on the cells is more sensitive and specific and is the gold standard test for diagnosis of PNH.[12]

Treatment of PNH is driven by the specific disease presentations. In most cases, treatment is essentially supportive, aiming at improving the quality of life. The only curative therapy is haematopoietic stem cell transplant.[6] Eculizumab, humanized monoclonal antibody against complement C5, has been effective in improving symptoms of the patients.[13]

Most studies regarding PNH are from Western populations. Disease biology and clinical presentations are different and distinctive for population groups, and can show noticeable differences in geographic prevalence around the world. The present study was designed with an aim to see the clinico-haematologic features, as well

 

as study complications like thrombosis, to classify these patients and evaluate the size of the PNH clone in the patients of Pakistan (Asian population) as so far there is lack of data on PNH in our region. This will help to diagnose and determine treatment protocols.

## Materials and methods

### Patients

This study was a cross-sectional analysis conducted in the Department of Haematology, Armed Forces Institute of Pathology, Rawalpindi, from January 2013 to December 2017. All patients were Pakistanis, of Asian origin, belonging to different ethnic groups and included Punjabis, Pashtuns, Sindhis, Balochis, Kashmiris and those from Gilgit Baltistan. Patients were of both genders and between the ages of 20-55years. All subjects were elaborately apprised about the study and written informed consent was obtained.

### Clinico-haematological parameters

Detailed history and complete physical examination was done. Symptoms and signs were noted. CBC, peripheral blood film and reticulocyte count was done. Coombs test, urine for haemosiderin, bilirubin levels and serum LDH were done. Urine was tested for haemoglobin and haemosiderin and Leucocyte Alkaline Phosphatase score was performed. Abdominal ultrasound was done for splenomegaly or any other compications. Bone marrow examination of all patients was done to look for any concomitant myelodysplastic syndrome or aplastic anaemia.

### Immunophenotyping

Diagnosis was established by flow cytometric analysis. One ml of whole blood sample was used for immunophenotyping by flow cytometry. Monoclonal antibodies against CD55 and CD 59 were obtained from Becton-Dickinson Biosciences USA. Two colour flow cytometry was performed on FACS Flow Cytometer (Becton-Dickinson Biosciences USA).

### Classification

Patients were classified according to the Working Diagnostic Classification of the International PNH Interest Group (I-PIG) into three categories.

A. Classic PNH – clinical and lab evidence of intravascular haemolysis but no evidence of another defined bone marrow abnormality

B. PNH in the setting of another specified bone marrow disorder-clinical and lab evidence of intravascular haemolysis but also have concomitantly a defined underlying bone marrow abnormality

C. Subclinical PNH (PNH-sc)–No clinical or laboratory evidence of haemolysis

### Statistical analysis

Collected data was entered and analyzed using SPSS version 20.

I. Quantitative variables i.e. age, haemoglobin, platelet count and absolute neutrophil counts have been presented by mean±SD.

II. Qualitative variables i.e. gender, sub-categories have been presented by frequency and percentage.

## Results

A total of 43 patients of PNH were analyzed. Median age was 28years (14-48 years). Out of 43 patients, males were 28(65%) while remaining 15(35%) were females.

The clinical features are presented in Table 1. The most common presenting feature was pallor, fatigue and dyspnea followed by fever/infections, jaundice, dark colored urine and abdominal pain. On investigations, haemoglobinuria was seen in 22(51%) patients. Five patients (11%) had impaired renal functions. The median duration of disease at diagnosis was 3.8 yrs (0.8-6.3yrs). Three (6.9%) patients presented with thrombosis. Of these, two patients had hepatic vein thrombosis and one patient had thrombosis of the portal and splenic vein.

**Table 1** Clinico-haematological Characteristics (n=43)

| **Patient Characteristics** | |
|---|---|
| Gender, male-n (%) | 28(65) |
| Meadian age-years (range) | 28(14-48) |
| **Clinical Features-n (%)** | |
| Pallor | 42(98) |
| Fatigue | 40(93) |
| Dyspnea | 29(67) |
| Fever/Infection | 15(35) |
| Dark coloured urine | 9(21) |
| Abdominal pain | 8(19) |
| jaundice | 12(28) |
| Renal insufficiency | 5(11) |
| Haemoglobunuria | 22(51) |
| Thrombosis | 3(6.9) |
| **Peripheral blood findings-n (%)** | |
| Anaemia alone | 20(47) |
| anaemia and thrombocytopenia | 6(14) |
| Anaemia and neutropenia | 2(4.7) |
| Pancytopenia | 15(35) |
| **Bone marrow biopsy-n (%)** | |
| Aplasia (<5%) | 3(7) |
| Hypocellular Bone marrow (6-49%) | 15(35) |
| Normo and Hypocellular Bone marrow (≥50%) | 25(58%) |

On blood counts, all patients had anaemia. Twenty (47%) patients presented with anaemia alone, 8 (19%) had bicytopenia while 15(35%) had pancytopenia at the time of presentation. Mean haemoglobin was 7.8±2.6g/dl, mean platelet count was 94±52.8x$10^9$/l and mean ANC was 2.9±1.3x$10^9$/l. The mean reticulocyte percentage was 5.8%. Investigations revealed mean LDH of 982U/l and a mean bilirubin level of 0.9mg/dl. Urine for haemosiderin was positive in 20(47%) patients. LAP score was low in all patients.

Bone marrow biopsy was done to assess the cellularity of the

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marrow and to rule out any concomitant disorder. The cellularity of the marrow is shown in Table 1. None of our patients had myelodysplastic syndrome (MDS) on bone marrow examination.

On flow cytometric analysis, the mean clone size was 42.8%. Regarding the PNH clone, clones larger than 50% were seen in 23(53%) patients while 17(39%) patients had a clone size between 10-50% and 3(8%) patients had a clone size of less than 10%.

These forty-three patients were divided into three clinical groups based on Working Diagnostic Classification of the International PNH Interest Group (I-PIG). 23(53%) patients belonged to the classic PNH. Eighteen (42%) patients were classified as having PNH in setting of another bone marrow disorder (aplastic anaemia) and 2 (4.7%) had sub-clinical PNH. The median ages of Classic PNH, PNH in the setting of a bone marrow disorder and sub-clinical PNH were 23, 29 and 32 years, respectively. We also evaluated the size of the PNH clone in each sub-category. The median clone size was 60% in patients with classic PNH, 22% in patients with PNH in setting of another bone marrow disorder and 7% in sub-clinical PNH as shown in Table 2. Table 3 shows the stratification of the patients in each subgroup according to clone size.

**Table 2** Classification of our PNH patients

| Classic PNH | PNH in setting of underlying disorder | Sub-Classical PNH | |
|---|---|---|---|
| No. of Patents-n (%) | 23(53%) | 18(42%) | 2(4.7%) |
| Median age-years | 23(13-36) | 29(24-48) | 32(23-40) |
| PNH clone size-% | 60(16-85) | 25(7-65) | 7(0-15) |

**Table 3** Stratification of the patients in each subgroup according to clone size

| Patents-n(%) | PNH Clone Sixe | | |
|---|---|---|---|
| | <10% | 10-50% | ≥50% |
| Classic PNH | - | 3(7%) | 20(46.5%) |
| PNH in setting of underlying disorder | 2(4.7%) | 13(30.2%) | 3(7%) |
| Sub-Clasical PNH | 1(2.3%) | 1(2.3%) | - |

## Discussion

Despite the fact that that PNH results from an acquired somatic mutation in the PIG-A gene, PNH patients show clinical heterogeneity and diversity. These patients have a highly variable clinical course with some patients having mild symptoms for years while others may present with life-threatening complications. Flow cytometric analysis has revolutionized the diagnosis of this rare disorder and is now the gold standard.

To our knowledge, there is no comprehensive data on the clinico-haematologic features, immunophenotypic profile and classification of PNH patients from our part of the country. Armed Forces Institute of Pathology is a tertiary care institute and referral center in north of the country. It caters to a large number of patients from all over the country from very different ethnic backgrounds. Our study aims to help clinicians in diagnosis and structuring treatment decisions in light of clone size.

In our study, the median age of the patients was 28years. A.P. De Azambuja et al.[14] have reported the age of the Brazilian patients as 24.1years. However, a much higher age, 42years, has been reported by Schrezenmeier et al.[15] in the Western population. Among our patients, males were more common as compared to females (65% vs 35%). The male to female ratio was 1.9:1. Similar findings have been reported by Nishimura et al.,[16] who has reported a gender ratio of 1.3:1 in the Japanese population. However, Wang et al.[17] and Schrezenmeier et al.[15] have reported female predominance.

The most common presenting clinical findings have been compared in Figure 1 with study conducted by A P De Azambuja et al.[14] in the Brazilian population. In our study population, 6.9% patients presented with thrombosis. These findings are consistent with those (5.6%) reported by Nishimura et al.[16] in the Japanese population. However, much higher frequencies of 16.5% and 16.7% have been reported by De Azambuja et al.[14] and Wang et al.,[17] respectively.

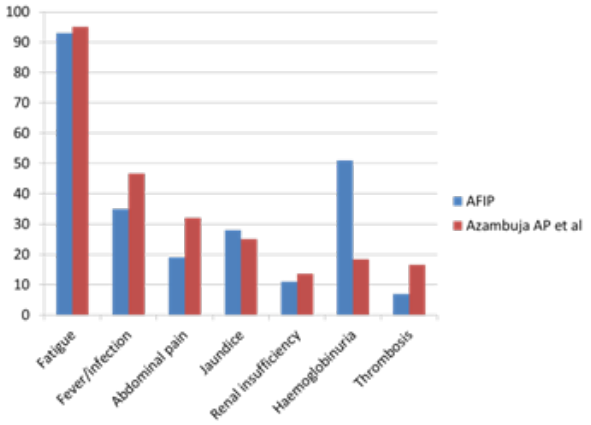


**Figure 1** Comparison of clinical presentation with International studies.

Our patients had median haemoglobin of 7.8g/dl at the time of presentation. These findings are in accordance with haemoglobin of 7.4g/dl reported by Wang et al.[17] However, Schrezenmeier et al.[15] have reported higher haemoglobin levels. As compared in Table 4, De Azambuja et al.[14] have reported low platelet counts as compared to our study. The reticulocyte percentage of our patients is comparable with that reported by Wang et al.[17]

We classified our patients into 3 subgroups. Twenty three (53%) patients belonged to the classic PNH. Eighteen (42%) patients were classified as having PNH in setting of another bone marrow disorder and 2(4.7%) had sub-clinical PNH. This has been compared to other international studies in Figure 2. The median ages of Classic PNH, PNH in the setting of a bone marrow disorder and sub-clinical PNH were 23, 29 and 32years, respectively in our study. However, De Azambuja et al.[17] has reported the median ages as 34.7, 21.5 and 25.1 respectively in the Brazilian population. On evaluation of the size of the PNH clone in each sub-category, the median clone size was 60% in patients with classic PNH, 22% in patients with PNH in setting of another bone marrow disorder and 7% in sub-clinical PNH. The median clone size reported by De Azambuja et al.[17] is 82.2% in classic PNH, 15.8% in PNH in setting of another bone marrow disorder and 0.04% in sub-clinical PNH.[18,19]

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**Table 4** Comparison of lab parameters with International studies

| AFIP | Wang et al.[17] | De Azambuja et al.[14] | Nishimura et al.[16] | Schrezenmeier et al.[15] |
|---|---|---|---|---|
| Haemoglobin(g/dl) | 7.8 | 7.4 | 8.8 | 10.6 |
| Platelets($X10^9$/l) | 104 | 97 | 25 | 131 |
| ANC($X10^9$/l) | 2.8 | - | 0.94 | 1.7 |
| Reticulocyte (%) | 5.8 | 5.1 | - | - |
| LDH (U/L) | 983 | 2920 | 328 | - |

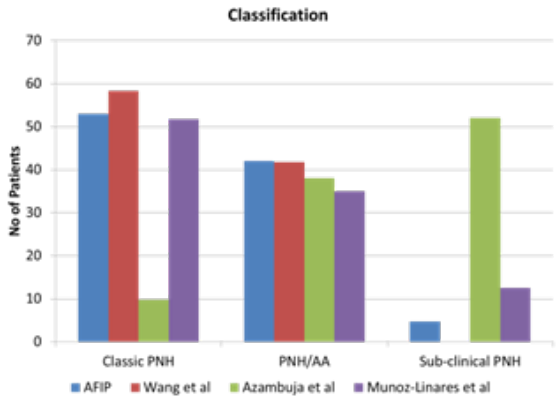


**Figure 2** Classification of PNH patients compared to International data.

## Conclusion

Paroxysmal nocturnal haemoglobinuria in Pakistan has not been studied extensively. Most of our patients presented as classic PNH followed by PNH in the setting of a bone marrow disorder. Thrombosis was rarely seen in our patients. However, larger studies are needed to study the natural history of the disease and the outcome of these patients.

## Acknowledgement

None.

## Ethical approval

This study was approved by the Ethical Review Committee of Armed Forces Institute of Pathology, Rawalpindi. Informed written consent was taken from the patients. Informed consent has been taken from all patients.

## Conflict of interest

Author's Disclosures of Potential Conflicts of Interest. No potential conflicts of interest relevant to this article were reported.

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